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Hybridization Among the Ancient Mariners: Characterization of Marine Turtle Hybrids With Molecular Genetic Assays

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Reports of hybridization between marine turtle species (family Cheloniidae) have been difficult to authenticate based solely on morphological evidence. Here we employ molecular genetic assays to document the sporadic, natural occurrence of viable interspecific hybrids between species representing four of the five genera of cheloniid sea turtles. Using multiple DNA markers from single-copy nuclear loci, eight suspected hybrids (based on morphology) were confirmed to be the products of matings involving the loggerhead turtle (*Caretta caretta*) × Kemp's ridley (*Lepidochelys kempii*) (*N* = 1 specimen), loggerhead turtle × hawksbill (*Eretmochelys imbricata*) (*N* = 2), loggerhead turtle × green turtle (*Chelonia mydas*) (*N* = 4), and green turtle × hawksbill (*N* = 1). Molecular markers from mitochondrial DNA permitted identification of the maternal parental species in each cross. The species involved in these hybridization events represent evolutionary lineages thought to have separated 10–75 million years ago (mya) and thus may be among the oldest vertebrate lineages capable of producing viable hybrids in nature. In some cases, human intervention with the life cycles of marine turtles (e.g., through habitat alteration, captive rearing, or attempts to establish new breeding sites) may have increased the opportunities for interspecific hybridization.

Anecdotal reports of marine turtle hybridization have circulated for decades, but scientific documentation has been elusive due in part to the overall morphological conservatism of this taxonomic group. Perhaps the earliest report of marine turtle hybridization involved the "McQueggie," alleged by Caribbean fishermen to be a hybrid between the loggerhead turtle (*Caretta caretta*) and the hawksbill turtle (*Eretmochelys imbricata*) (Garman 1888).

In the century since Garman's (1888) report, several sea turtle hybrids have been described, including offspring of *Chelonia mydas* × *E. imbricata* (Wood et al. 1983), *Ca. caretta* × *E. imbricata* (Frazier 1988; Kamezaki 1983), and *Ch. mydas* × *Ca. caretta* (Limpus C, personal communication). These appraisals were generally the result of serendipitous observation rather than systematic survey and were based on intermediate features in otherwise diagnostic morphologic characters. The first biochemical assay employed to identify hybrid marine turtles was protein electrophoresis, applied by Wood et al. (1983) to confirm the *Chelonia* × *Eretmochelys* cross noted above, and by Conceicão et al. (1990) to confirm a diagnosis of *Caretta* × *Eretmochelys* hybrids in Brazil.

Cases of marine turtle hybridization are remarkable because fossil and genetic data indicate that these species diverged from a common ancestor at least 10 million years ago (mya). In particular, the tribes Carettini (represented by *Caretta, Lepidochelys,* and *Eretmochelys*) and Chelonini (represented by *Chelonia*) are believed to reflect an ancient phylogenetic division within the family Cheloniidae, dating to perhaps 50+ mya (Bowen et al. 1993, and references therein).

In the course of population and evolutionary genetic analyses of marine turtles (Bowen et al. 1992, 1993, in press, and unpublished data; Karl et al. 1992), we developed a series of mitochondrial (mt) DNA and single copy nuclear (scn) DNA markers that can distinguish extant marine turtle species using restriction site polymorphisms (RSPs) and/or DNA sequence assays. The diploid scnDNA markers can identify the parental species of suspected hybrids and indicate whether such hybrids are probable first, or later, generation products. Under the assumption of maternal inheritance, the mtDNA assays indicate which species is the ma-

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Table 1. Summary of genetic analyses of putative sea turtle hybrids

Hybrid individual	Collection site	mtDNA genotype	scnDNA genotype
Hy1 and Hy2[a]	U.S.A.	CC	EI/CC
Hy3-Hy6[b]	Brazil	CC	CM/CC
Hy7[c]	U.S.A.	LK	CC/LK
Hy8[d]	Suriname	CM	EI/CM

[a] Clutch-mates from Southern Florida (L. Ehrhart and S. Johnson).

[b] Clutch-mates from the state of Bahia (Marcovaldi M, personal communication).

[c] Collected as a juvenile in Chesapeake Bay (Keinath J, personal communication).

[d] Collected as a single clutch by the Cayman Turtle Farm (Wood et al. 1983).

Species designations of the respective genotypes are as follows: CC = *Caretta caretta*; LK = *Lepidochelys kempii*; CM = *Chelonia mydas*; EI = *Eretmochelys imbricata*.

Table 2. Summary of nuclear DNA restriction profiles observed in four species and five putative hybrids of marine turtles

		Specimen							
Locus	Enzyme	CM	EI	LK[a]	CC	Hy1-2	Hy3-6	Hy7	Hy8
Cm-12	*Bgl* II	A	A	B	B	A&B	A&B	B	A
	Dde I	A	B	B	B	B	A&B	B	A&B
	Dra I	A	B	A	A	A&B	A	A	A&B
	Rsa I	A	A	B	C	A&C	A&C	B&C	A
Cm-14	*Alu* I	A	B	C	B	B	A&B	B&C	A&B
	Dra I	A	B	C	B	B	A&B	B&C	A&B
	Hae III	A	A	B	C	A&C	A&C	B&C	A
Cm-28	*Ava* II	A	B	—	B	—	A&B	—	A
	Bst NI	A	B	—	C	—	A&C	—	A
	Cfo I	A	B	—	B	—	A&B	—	A
	Dde I	A	B	—	C	—	A&C	—	A
Cm-39	*Bst* EII	A	—	—	B	—	A&B	—	—
	Dde I	A	—	—	B	—	A&B	—	—
Cm-45	*Nsi* I	A	B	—	B	—	A&B	—	A&B
	Sac I	A	B	—	B	—	A&B	—	A&B

[a] *Lepidochelys olivacea* and *L. kempii* produced identical banding patterns for all enzymes at all nuclear loci surveyed. Each identifiable restriction profile is assigned a different letter designation. A dash indicates that the enzyme was not surveyed. Species designations are as in Table 1.

ternal parent. Taken together, nuclear and mitochondrial assays afford a relatively thorough genetic assessment of putative hybrids.

Materials and Methods

Specimen Collection

Samples for this study were either tissues from hatchlings or blood taken from juveniles that appeared to be intermediate in putative parental species characteristics. Total cell DNA was isolated with a standard phenol-chloroform protocol (Herrman and Frischauf 1987). All DNAs were stored at 4°C in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Table 1 provides information on the collection sites of the individuals that proved to be interspecific hybrids.

Mitochondrial Genotype Determination

For each specimen, either RSP patterns or cytochrome *b* sequences from mitochondrial DNA were used to assign a maternal parent species. When fresh tissue samples were available (from live eggs or hatchlings), RSP analyses were conducted on purified mtDNA isolated by CsCl density gradient centrifugation (Lansman et al. 1981). Aliquots of purified mtDNA were digested with at least 11 of the 17 informative restriction enzymes listed in Bowen et al. (1992). Digestion fragments were end-labeled with ^{35}S nucleotides and separated on 1.0% agarose gels. Restriction fragments were visualized with autoradiography and assigned molecular weights by comparison to a 1-kb size standard (Gibco-BRL Inc.). Restriction profiles were compared to known fragment patterns from the six Cheloniid species (Bowen 1992, unpublished data).

In cases where the available tissues were frozen or partially degraded, we used mtDNA cytochrome *b* sequences to identify the maternal parent. Biotinylated versions of the primers described by Kocher et al. (1989) were used to amplify mtDNA sequences via the polymerase chain reaction (PCR) (Innes et al. 1990). To facilitate the automated sequencing protocol, M13 oligonucleotides complementary to standard sequencing primers were appended to the 5′ ends of these primers. Standard precautions, including the use of negative controls (template-free PCR reactions), were taken to guard against template contamination and related problems. PCR products were purified with streptavidin-coated magnetic particles (Promega). Single-stranded sequencing reactions were conducted with fluorescently labeled M13 primers in a robotic work station (Applied Biosystems Model 800), and the labeled extension products were analyzed with an automated DNA sequencer (Applied Biosystems Model 373A) in the DNA Sequencing Core at the University of Florida. Approximately 200 bps of mtDNA cytochrome *b* sequence from the putative hybrids were compared to known sequences from the six Cheloniid species (Bowen et al. 1993). In all cases, the species assignments were unambiguous, because both the RSP and cytochrome *b* assays of mtDNA included a minimum of five species-diagnostic characters.

Nuclear Genotype Determination

Genotypes from single copy nuclear DNA were determined following the procedure of Karl et al. (1992; see also Karl and Avise 1993). Five of the anonymous scnDNA primers (Cm-12, Cm-14, Cm-28, Cm-39, and Cm-45) previously developed for the green turtle (*Ch. mydas*) also amplified homologous regions from six of the seven marine turtle species. The exception was the leatherback turtle, *Dermochelys coriacea* (sole member of the family Dermochelyidae), whose DNA failed to amplify with these five primers under a variety of conditions (Karl, unpublished data). Using these primers, the DNA from one individual of each of the six species was amplified and screened with up to 40 endonucleases (4-, 5-, and 6-base pair recognition sequences) to identify species-characteristic restriction profiles. Once suitable primer/enzyme combinations were determined, the target loci from suspected hybrids were amplified and digested with the taxonomically informative enzymes. Fragment digestion profiles were assayed using 2% agarose gels stained with ethidium bromide.

Initially, only one individual from each species was assayed for informative markers (with the exceptions noted below), so there was a possibility that intraspecific polymorphisms could compromise the species assignments. To circumvent this problem, several putative species-characteristic restriction sites were surveyed in each hybrid. Agreement in genotypic assignment at most or all enzyme sites provided an internal control for species identification (in other words, a species diagnosis would unlikely be compromised by simultaneous polymorphisms at more than one restriction site, particularly for genetically distinct species and genera [Bowen et al. 1993]). Furthermore, for

green turtles and loggerheads (where much larger sample sizes were available), the degree of intraspecific polymorphism at the restriction sites employed as markers was known to be highly constrained (Karl et al. 1992, unpublished data), an observation that further supports our confidence in the species assignments in the current study.

Results

Due to the diversity of genetic assays and the occasional complexities of interpretations, results will be presented separately for each hybrid class identified. The overall results are summarized in Tables 1 and 2.

Chelonia × *Caretta*

During the course of global population genetic surveys of green and loggerhead turtles (>175 individuals assayed from each species; Bowen et al. 1992, in press), four hatchling clutch-mates from Brazil (henceforth Hy3–Hy6), originally thought to be green turtles, unexpectedly displayed loggerhead mtDNA restriction profiles upon digestion with 11 informative enzymes (Figure 1). Subsequently, ~200 bps of the cytochrome *b* gene from Hy3 were assayed, and these included five nucleotides normally diagnostic for loggerheads.

All four individuals also were screened at several scnDNA loci (Figure 2), using two to five restriction enzymes per locus, which when considered in combination produced species-characteristic gel patterns [Cm-12 (*Bgl* II, *Dde* I, *Dra* I, and *Rsa* I); Cm-14 (*Alu* I, *Dra* I, and *Hae* III); Cm-28 (*Ava* II, *Bst* NI, *Cfo* I, and *Dde* I); Cm-39 (*Bst* EII and *Dde* I); and Cm-45 (*Nsi* I and *Sac* I)]. Although no single enzyme produced unique restriction profiles for all species, the multiple enzyme patterns allowed the species identity to be determined unequivocally (Table 2). For example, the digestion of locus Cm-12 with *Rsa* I produced a restriction profile unique for loggerhead turtles, but a shared pattern for green turtles and hawksbills (the *Lepidochelys* banding pattern is also unique with this enzyme and was not observed in Hy3–Hy6), whereas at this same locus, digestion with *Dde* I produced a restriction profile unique for green turtles, but patterns otherwise nondiagnostic for the remaining turtle species. Thus, by considering the profiles for both enzyme digests jointly, one allele at the Cm-12 locus must have come from a loggerhead turtle and the other from a green turtle. Using similar reasoning, digestion profiles from Cm-14 for each of these individuals appeared to be a composite of green turtle and loggerhead turtle alleles. Three other scnDNA loci (Cm-28, Cm-39, and Cm-45) were surveyed in green turtles and loggerheads. The restriction profiles for green turtles were different from the loggerheads with all enzymes surveyed. Because not all marine turtle species were assayed, exact species assignments could not be made at these loci. Nonetheless, all digestion profiles were consistent with the results from Cm-12 and Cm-14. These findings, in conjunction with the mtDNA evidence, indicate that these hatchlings were F_1 hybrids between a loggerhead female and a green turtle male.

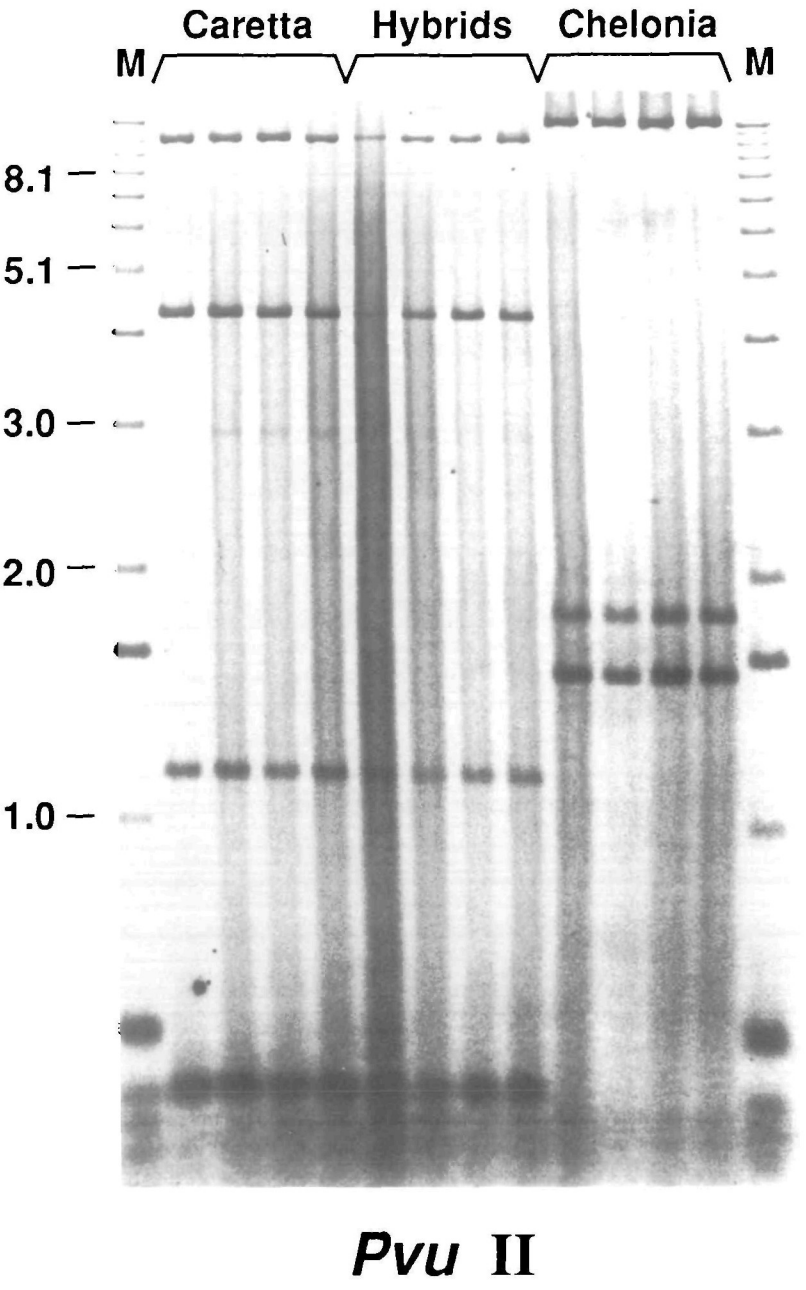

Figure 1. Mitochondrial DNA restriction fragment profiles produced by digestion with *Pvu* II for four specimens each of *Caretta caretta* (lanes 2–5), *Chelonia mydas* (lanes 10–13), and presumptive hybrids between these species from Brazil (Hy3–Hy6, Table 1). Lanes 1 and 14 are molecular weight standards with the sizes (in kb) of selected fragments indicated.

Caretta × *Eretmochelys*

Based on morphological appearance, two clutch-mates (henceforth Hy1 and Hy2, collected in Florida and maintained in captivity at Sea World in Orlando, Florida) were suspected of being hybrid derivatives of a cross between a loggerhead turtle and a hawksbill. Cytochrome *b* se-

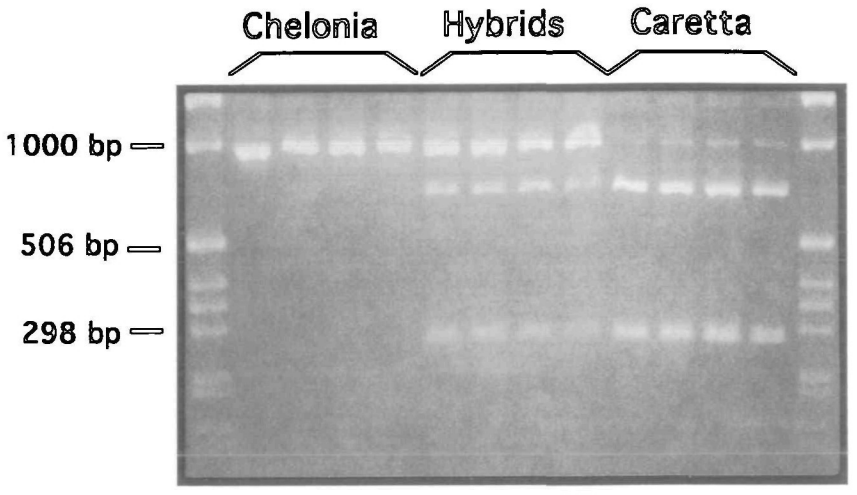

Figure 2. Nuclear DNA restriction fragment profiles (Cm-45 cut with *Sac* I) for four specimens each of *Caretta caretta* (lanes 10–13), *Chelonia mydas* (lanes 2–5), and presumptive hybrids involving *Caretta caretta* × *Chelonia mydas* (Hy3–Hy6, Table 1). Lanes 1 and 14 are molecular weight standards with the sizes (in bp) of selected fragments indicated.

quences from these individuals included six nucleotides normally diagnostic for loggerhead turtles, thus indicating that the maternal lineage was *Ca. caretta.*

Nuclear DNA from these individuals was of marginal quality and did not amplify well for several of the scnDNA loci. Nonetheless, *Hae* III digestions at the Cm-14 locus indicated that these individuals were hybrids between a loggerhead turtle and either a green turtle or a hawksbill (but not a ridley—*Lepidochelys olivacea* or *L. kempii*) and a *Dra* I digestion eliminated the green turtle as a possible parent. In a similar fashion, digestions at the Cm-12 locus with two enzymes (*Dra* I and *Rsa* I) unequivocally identified both Hy1 and Hy2 as loggerhead × hawksbill hybrids. Taken together, the genetic and morphological data indicate that these two specimens are F_1 hybrids between a loggerhead female and a hawksbill male.

Caretta × *Lepidochelys*

During the summer of 1992, J. Keinath and J. Musick (Virginia Institute of Marine Science) identified a suspected loggerhead × Kemp's ridley hybrid turtle (Hy7) in Chesapeake Bay. Blood drawn from this individual was the source for cytochrome *b* sequence analysis of mtDNA and for RSP analysis of scnDNA. The cytochrome *b* sequence revealed four nucleotides diagnostic for the genus *Lepidochelys,* and two additional sites unequivocally identified the specimen as having a Kemp's ridley (*L. kempii*) maternal lineage.

Only two nuclear loci were screened, both indicating that Hy7 was a ridley × loggerhead hybrid. Restriction digests at the Cm-12 (*Bgl* II, *Dde* I, *Dra* I, and *Rsa* I) and Cm-14 (*Alu* I, *Dra* I, and *Hae* III) loci produced gel profiles that combined fragments otherwise characteristic for samples of *Caretta* versus *Lepidochelys* (including a 25-bp size polymorphism at locus Cm-14, unique in our samples to the two species of ridley turtles). In summary, Hy7 appeared to be the F_1 product of a cross involving a Kemp's ridley female and a loggerhead male.

Chelonia × *Eretmochelys*

Since 1977, Cayman Turtle Farm (Grand Cayman Island) has maintained 37 turtles (originally collected in Suriname) that were suspected of being hybrids between green turtles and hawksbills. In captivity, these individuals have been observed to mate with green turtles and are known to possess motile sperm. Blood was collected from one of these specimens (Hy8) and subjected to genetic analysis. Cytochrome *b* sequencing revealed five nucleotides characteristic of green turtle mtDNA.

Analyses with scnDNA loci revealed a complex pattern. For two loci, Cm-12 and Cm-14, enzyme digestions (*Bgl* II, *Dde* I, *Dra* I, and *Rsa* I; *Alu* I, *Dra* I, and *Hae* III, respectively) produced restriction fragment profiles indicating that this specimen was unequivocally a green turtle × hawksbill hybrid. Digestion profiles for Cm-45 (*Nsi* I and *Sac* I) were consistent with one of the parents being a green turtle and the other parent being either a hawksbill or a loggerhead. The inability in this case to distinguish between the alternatives for the second parent is due to a lack of a known species-specific restriction profile, which can distinguish hawksbills from loggerheads at this locus. One locus (Cm-28) produced only the green turtle restriction pattern after digestion by four enzymes (*Ava* II, *Bst* NI, *Cfo* I, *and Dde* I), and Cm-39 was not screened. The failure of Cm-28 to confirm the hybrid status for this individual raises the possibility that the specimen is a second generation (or later) hybrid derived from backcross(es) to green turtles. Alternatively, the primers could have failed to amplify adequately from the heterologous loggerhead template.

Discussion

The hybridization events documented here involve four of the five Cheloniid genera and four of the six Cheloniid species. Of the remaining species, the olive ridley (*Lepidochelys olivacea*) is reported to hybridize with green turtles in Brazil (Marcolvaldi M, personal communication), but specimens were unavailable for genetic analysis at the time of this writing. An absence of documented hybrids involving the flatback turtle (*Natator depressus*) may be due in part to its limited range in Australia and New Guinea.

Ages of Hybridizing Lineages

Marine turtles (superfamily Chelonioidea) apparently diverged from an ancestral form ~150 mya, and the family Cheloniidae may have separated from the Dermochelyidae (extant genus *Dermochelys*) at least 100 mya (Weems 1988; Zangerl and Sloan 1960). Within Cheloniidae, the two tribes Chelonini (*Chelonia*) and Carettini (*Caretta, Eretmochelys,* and *Lepidochelys*) have been separated for perhaps 50–75 million years (Bowen et al. 1993; Ernst and Barbour 1989; but see Zangerl 1980), whereas lineages leading to the latter three genera probably separated on the order of 10–20 mya (Dodd and Morgan 1992; Zangerl 1980). Based on these provisional dates as determined primarily from fossil evidence, the average time of separation for the hybridizing lineages in this report is at least 30 mya, and the oldest estimates involve species separated for more than 50 million years.

How do these values compare to other age records for vertebrate hybridization? From surveys of species known to be capable of producing viable hybrids, Wilson et al. (1974) reported that the *oldest* such hybridizing lineages among mammalian taxa involved species separated for ~6 million years. From similar genetic evi-

Table 3. Estimated times of phylogenetic separation for various vertebrate species known to be capable of producing viable hybrids

Lineages involved in hybrid cross	Time since separation (mya)[a]	Number of individuals	Reference
Caretta caretta/Eretmochelys imbricata	10–20	2[b]	Current study
Chelonia mydas/Eretmochelys imbricata	50+	1	Current study
Chelonia mydas/Caretta caretta	50+	4[b]	Current study
Lepidochelys kempii/Caretta caretta	10–20	1	Current study
Various mammals	3 (max. 6)	—	Wilson et al. 1974
Various birds	20 (max. 57)	—	Wilson et al. 1974
Various amphibians	20 (max. 54)	—	Prager and Wilson 1975

[a] For the marine turtles in this report, estimated dates come primarily from fossil evidence, but are in part corroborated by molecular data (Bowen et al. 1993, and references therein). Separation times for the mammals, birds, and amphibians are from the references indicated and citations therein.

[b] Turtles from the same clutch.

dence, the average age of hybridization-capable lineages within both birds and frogs was estimated to be ~20–25 million years (Prager and Wilson 1975; Wilson et al. 1974), with a few taxa reportedly having retained the evolutionary capacity for viable hybrid production for slightly more than 50 million years (Table 3). Thus, avian and amphibian taxa apparently lose the capacity for hybrid production much more slowly over evolutionary time than do mammals. To our knowledge, the most ancient lineages known to be capable of producing viable hybrids involve frogs of the genera *Hyla* and *Pseudacris* (Wilson et al. 1974), which may have been isolated for perhaps 80 million years by the separation of Africa from South America.

However, many of the above-mentioned hybridizations involve crosses conducted under artificial or forced conditions in the laboratory. Therefore, the Carettini and Chelonini (*Chelonia* × *Caretta,* and *Chelonia* × *Eretmochelys*) crosses may be the oldest vertebrate lineages known to hybridize in nature.

What accounts for the capacity of these ancient mariners to produce viable hybrids? Several studies have suggested that turtle mtDNA evolves slowly relative to the conventional mtDNA clock for vertebrates (Avise et al. 1992), and this conclusion may apply to scnDNA (Karl and Avise 1993; Karl et al. 1992) and microsatellite loci as well (FitzSimmons et al. 1995). Chromosomal evolution is known to be extremely slow in marine turtles (Bickham 1981), and the reduced pace of anatomical evolution of turtles is a proverbial feature of the group. These observations prompt the conclusion that a low evolutionary rate (including the loss of interspecific hybridization potential) is a pervasive feature of marine turtle genetics and evolution.

Perhaps a conservative pattern of genomic evolution is a necessary prerequisite to hybridization between relatively ancient turtle lineages. The maintenance of chromosomal number and structure (Bickham 1981) is one indication of potential genomic compatibility, but additional genetic features no doubt are also involved in the development of viable hybrids. Prager and Wilson (1975) suggest that evolutionary changes in genetic regulatory systems are the primary basis for loss of hybridization potential. In other words, differences in the pattern of gene expression (rather than in the makeup of structural genes) may contribute most to the physiological and ontogenetic basis of hybrid inviability. Data presented here are consistent with such conclusions but do not permit a critical appraisal.

Frequencies of Interspecific Hybridization

The nesting habitats of marine turtle species overlap extensively in every tropical and temperate ocean basin, occasionally with three or more species sharing a nesting beach. Hence, opportunities for hybridization may abound, yet reported instances of hybrids are few. One possibility is that such cases are underreported. When preliminary molecular data on hybrid turtles was presented at the 12th annual marine turtle symposium in 1992 (Richardson and Richardson 1995), many participants responded with anecdotal reports of "unusual hatchlings," which in retrospect may have been hybrids. For example, some captive-hatched "green turtles" at the Cayman Turtle Farm have "*Eretmochelys*-like" morphology (Wood F, personal communication), and, hatchlings representing a presumed *Chelonia* × *Caretta* cross (at a Queensland, Australia rookery during the 1990–1991 nesting season) have been maintained in captivity for several years (Limpus C, personal communication).

In discussions of hybridization frequency, an important point is that a clear distinction must be made between interspecific matings themselves and successful hybridization events that result in production of viable progeny. For example, our data cannot rule out the possibility that interspecific matings are relatively common in areas of overlap, but that viable offspring are produced only occasionally. The fate of marine turtles hybrids in the wild is extremely difficult to document. However, some clues may be assembled from observations on captive specimens at the Cayman Turtle Farm. Hybrid offspring from the *Chelonia* × *Eretmochelys* cross described by Wood et al. (1983, personal communication) had high mortality and were more susceptible to lung infection than captive green turtles, suggesting that crosses between species representing Chelonini and Carettini have reduced fitness. On the other hand, this intertribe cross produced at least one male who survived to maturity, had motile sperm, and was observed to mate with resident green turtle females (Wood F, personal communication).

Gender-Based Aspects of Hybridization

The mating behaviors of marine turtles are poorly understood (but see Limpus 1993). Most species appear to have distinct courting areas in the vicinity of nesting beaches. From commonalities among the four cases of hybridization documented here, two generalities may be postulated. First, the marine turtle hybrids all occurred in regions of shared nesting range. Therefore, a temporal and spatial overlap in mating areas probably facilitates and may even be necessary for hybridization (Conceicão et al. 1990; Wood et al. 1983). Second, all documented instances of hybridization occurred in locations where one species is abundant and the other relatively rare. Perhaps a scarcity of mates for one species enhances the likelihood of interspecific couplings, as, for example, has previously been suggested for genetically documented hybridizations among species of sunfish in Georgia (Avise and Saunders 1984). However, in both cases (sunfish and marine turtles), this latter observation may be an artifact of the relatively small numbers of hybrids discovered and of the fact that areas of species overlap typically are characterized by highly unequal species densities.

In another respect, the hybridization re-

sults for the sunfish and the marine turtles differed. Among the former, there was a strong tendency (six of the seven reported instances) for the rare species in a hybrid cross to provide the female parent. This observation suggests that an absence of conspecific pairing partners and/or spawning stimuli for females of rarer species might be important factors increasing the likelihood of interspecific hybridization (Avise and Saunders 1984). By contrast, in three of the four hybrid crosses presently reported for marine turtles (*E. imbricata* ♂ × *Ca. caretta* ♀, *Ch. mydas* ♂ × *Ca. caretta* ♀, and *E. imbricata* ♂ × *Ch. mydas* ♀), the female parent came from the locally abundant species. In the fourth instance of marine turtle hybridization, the female parent *L. kempii* was exceedingly rare relative to the male parent *Ca. caretta,* but this may be a special case because the reciprocal cross may be prohibited for mechanical reasons (see below). Notably, *Ca. caretta* served as either a male or a female parent in these hybridizations, so no inherent gender bias in the directionality of crosses was apparent.

A possible tendency for hybridizing females to represent common as opposed to rare species in marine turtles may be attributable in part to the general mating proclivities of the two genders. Male marine turtles are somewhat indiscriminate in mating preferences. At some breeding locations, fishermen routinely capture male green turtles by placing barrels or other crude decoys in the water (Carr 1956). Males will mount decoys in stereotypical breeding behavior and remain attached while the decoy is retrieved. As is true for many other animal species, female marine turtles may be the more discriminating gender in mate selection and would thereby represent the limiting factor in interspecific couplings. With more females available from common species, a constant "error" rate in mate choice per female (heterospecific matings) could translate into a numerical predominance of hybrids with mothers from the more common species.

Nonetheless, additional gender-based considerations likely come into play. For example, some interspecific matings may be difficult or impossible for simple mechanical reasons. Male turtles are equipped with a large claw on each front flipper and must firmly grasp the anterior margin of the female's shell for copulation. Males who are missing front flippers or otherwise cannot secure the female are generally unable to mate (Limpus 1993). Hence, males of a smaller species (such as *L. kempii*) may be unable to copulate with females from a larger species (such as *Ca. caretta* or *Ch. mydas*) for purely mechanical reasons. In the single case of hybridization in this study involving species differing greatly in size (*Ca. caretta* × *L. kempii*), the female parent was indeed from the smaller (Kemp's ridley) species (Table 1).

Hybridization Issues and Marine Turtle Conservation

All species of marine turtles are formally listed as threatened or endangered (IUCN 1993). In recent decades, many management programs for marine turtles have attempted transplantations and captive rearing to enhance natural populations (Mrosovsky 1983). In the case of Kemp's ridley, in the years 1978–1992, >18,000 eggs were transplanted from Tamaulipas, Mexico to Padre Island, Texas, and the hatchlings were reared ("headstarted") in captivity before release. In recent years, some unusual behaviors have been observed for this species in nature, including reproductive activity on the Atlantic coast, some 2,000 km outside the historical nesting range of *L. kempii* (Bowen et al. 1994). The possibility exists that this previously unnoticed nesting behavior may be attributable to modification of early life-history stages via the captive rearing program at Padre Island (Bowen et al. 1994). In this regard, it is noteworthy that the Kemp's ridley × loggerhead hybrid identified in the current study was recovered on the mid-Atlantic coast in 1992 (Frank 1992).

Generalizations about the impact of hybridization on natural populations are difficult to make, but other cases exist in which human-directed transplantation schemes have led to decline and extinction of endangered taxa (Templeton 1994). It is therefore imperative that wildlife managers consider such possibilities before manipulating the natural histories of endangered species.

References

Avise JC and Saunders NC, 1984. Hybridization and introgression among species of sunfish (Lepomis): analysis by mitochondrial DNA and allozyme markers. Genetics 108:237–255.

Avise JC, Bowen BW, Bermingham E, Meylan AB, and Lamb T, 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Mol Biol Evol 9:457–473.

Bickham JW, 1981. Two-hundred-million-year-old chromosomes: deceleration of the rate of karyotypic evolution in turtles. Science 212:1291–1293.

Bowen BW, 1992. Evolutionary genetics and natural history of marine turtles (PhD dissertation). Athens: University of Georgia.

Bowen BW, Conant TA, and Hopkins-Murphy SR, 1994. Where are they now? The Kemp's ridley headstart project. Conserv Biol 8:853–856.

Bowen BW, Nelson WS, and Avise JC, 1993. A molecular phylogeny for marine turtles: trait mapping, rate assessment, and conservation relevance. Proc Natl Acad Sci USA 90:5574–5577.

Bowen BW, Kamezaki N, Limpus CJ, Hughes GH, Meylan AB, and Avise JC, in press. Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. Evolution.

Bowen BW, Meylan AB, Ross JP, Limpus CJ, Balazs GH, and Avise JC, 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865–881.

Carr A, 1956. The Windward Road. New York: Knopf.

Conceicão MB, Levy JA, Marins LF, and Marcovaldi MA, 1990. Electrophoretic characterization of a hybrid between *Eretmochelys imbricata* and *Caretta caretta* (Cheloniidae). Comp Biochem Physiol 97B:275–278.

Dodd CK Jr and Morgan GS, 1992. Fossil sea turtles from the early Pliocene bone valley formation, central Florida. J Herpetol 26:1–8.

Ernst CH and Barbour RW, 1989. Turtles of the world. Washington, D.C.: Smithsonian Institution Press.

FitzSimmons NN, Moritz C, and Moore SS, 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol Biol Evol 12:432–440.

Frank J, 1992. Hybrid sea turtle found in net: discovery by fisherman called "1 in a billion." Virginian Pilot Tues July 21.

Frazier J, 1988. Sea turtles in the land of the dragon. Sanctuary (Asia) 8:15–23.

Garman S, 1888. Reptiles and batrachians from the Caymans and the Bahamas. Bull Essex Inst 20:1–13.

Herrman B and Frischauf A, 1987. Isolation of genomic DNA. Methods Enzymol 152:180–183.

Innes MA, Gelfand DH, Sninsky JJ, and White TJ, 1990. PCR protocols: a guide to methods and applications. San Diego: Academic Press.

IUCN (World Conservation Union), 1993. 1994 IUCN red list of threatened animals. Gland, Switzerland: IUCN.

Kamezaki N, 1983. The possibility of hybridization between the loggerhead turtle, *Caretta caretta,* and the hawksbill turtle, *Eretmochelys imbricata,* in specimens hatched from eggs collected in Chita Peninsula. Jpn J Herpetol 10:52–53.

Karl SA and Avise JC, 1993. PCR-based assays of Mendelian polymorphisms from anonymous single copy nuclear DNA: techniques and applications for population genetics. Mol Biol Evol 10:342–361.

Karl SA, Bowen BW, and Avise JC, 1992. Global population structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analysis of anonymous nuclear DNA loci. Genetics 131:163–173.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, and Wilson AC, 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86:6196–6200.

Lansman RA, Shade RO, Shapira JF, and Avise JC, 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J Mol Evol 17:214–226.

Limpus C, 1993. The green turtle, *Chelonia mydas,* in Queensland: breeding males in the southern Great Barrier Reef. Wildl Res 20:513–523.

Mrosovsky N, 1983. Conserving sea turtles. London: British Herpetological Society.

Prager EM and Wilson AC, 1975. Slow evolutionary loss of the potential for interspecific hybridization in birds: a manifestation of slow regulatory evolution. Proc Natl Acad Sci USA 72:200–204.

Richardson JI and Richardson TH, 1995. Proceedings of the twelfth annual workshop on sea turtle biology and conservation. NOAA technical memo (NMFS-SEFSC-361). Springfield, Virginia: National Technical Information Service.

Templeton AR, 1994. Coadaptation, local adaptation, and outbreeding depression. In: Principles of conservation biology (Meffe GK and Carroll CR, eds). Sunderland, Massachusetts: Sinauer; 152–153.

Weems R, 1988. Paleocene turtles from the Aquia and Brightseat formations, with a discussion of their bearing on sea turtle evolution and phylogeny. Proc Biol Soc Wash 101:109–145.

Wilson AC, Maxson LR, and Sarich VM, 1974. Two types of molecular evolution. Evidence from studies of interspecific hybridization. Proc Natl Acad Sci USA 71:2843–2847.

Wood JR, Wood FE, and Critchley K, 1983. Hybridization of *Chelonia mydas* and *Eretmochelys imbricata*. Copeia 1983:839–842.

Zangerl R, 1980. Patterns of phylogenetic differentiation in the Toxochelyid and Cheloniid sea turtles. Am Zool 20:585–596.

Zangerl R and Sloan RE, 1960. A new specimen of *Desmatochelys lowi* Williston; a primitive Cheloniid sea turtle from the Cretaceous of South Dakota. Fieldiana Geol 14:7–40.

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